

VIRUCIDAL ACTION OF HYDROGEN PEROXIDE
AEROSOLS IN DECONTAMINATION OF AIR IN
AN INFLUENZA NIDUS

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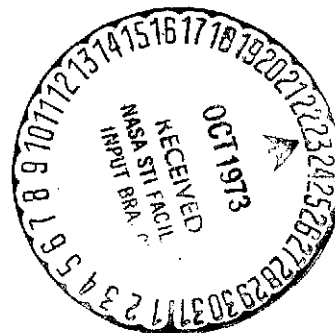
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16. Abstract Hydrogen peroxide aerosols were used for decontamination of the atmosphere in premises of an influenza epidemic nidus. The work was carried out in a children institution during the influenza outbreak. Influenza virus, type A ₂ (strain Hong-Kong-68) was isolated from 12 out of 27 air samples tested before the disinfection. A distinct virucidal effect (40 min. and 3 hours after the treatment no influenza virus was isolated from the air) followed a single application of hydrogen peroxide aerosols (0.020-0.031 g of the preparation per cubic meter of the premises). In the premises which were only ventilated the influenza virus of the same type was isolated in 6 air samples taken in the course of one day. The use of hydrogen peroxide aerosol is the most expedient for decontamination of the atmosphere in premises in which human beings stay for short periods of time.			
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VIRUCIDAL ACTION OF HYDROGEN PEROXIDE AEROSOLS IN
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B.P. Fedayayev, K.M. Florensova, V.V. Sidenko and Z.I. Stepanova

The methods of immunoprophylaxis which are employed do not /137*
always prevent the spread of such massive infections as flu and
other acute respiratory diseases which periodically take on the
character of epidemic outbreaks. Therefore, it is justifiable
to devise measures of nonspecific prevention of these infections.
One of the most effective measures is the disinfection of the air.
Among the measures of combating flu, timely and effective decon-
tamination of the air in public places and areas used for different
purposes together with other antiepidemic measures may play an
important role in preventing the disease.

A number of investigators have found that the agents of flu
retain their viability in the environment for various periods of
time depending on the conditions. Merekalova (1956), Dmitriyeva
(1957) and others have found the flu virus in the air of certain
locations in the course of 4 1/2 to 6 hours, Balan (1969) found
flu agents after 20 to 22 hours with 58-60% relative humidity of
the air in an experimental chamber, and Val'vechev et al. (1967)
found the virus in the vicinity of flu patients on objects in the
environment for 1 to 14 and even 30 days.

Numerous studies have been devoted to searching for chemical,
physical and mechanical methods and means of decontaminating air
with respect to flu, but two of the latest methods do not always

*Numbers in the margin indicate pagination in original foreign text.

correspond to the epidemiological requirements for conditions of periodic decontamination of the air of rooms. Of all the chemical substances that can be used for disinfecting the air with spot infections, it has been suggested that the following preparations be used in the form of solutions, vapors and aerosols: chlorine-containing substances (Merekolova, 1956), galactic acid (Vashkov and Astaf'yeva, 1954), triethylene glycol (Ginsburg, 1946), hexyl-resorcine (Vashkov et al., 1954) and hydrogen peroxide (Val'vachev et al., 1967) and others. However, not all of these substances are harmless to the organism or convenient to use. The attention of investigators has been attracted in particular to hydrogen peroxide as a disinfectant: in concentrations used for decontamination, it will not harm the object being treated, has no odor, breaks down rapidly and has a low level of toxicity for warm-blooded animals. In the opinion of a number of authors (Shumayeva, 1966; Val'vachev et al., 1967), the low toxicity of hydrogen peroxide solutions makes it possible to use them for decontamination of rooms when human beings are present.

We know (Tsetlin and Vil'kovich, 1965) that it is most advantageous and economical to use disinfectants as aerosols with a certain degree of dispersity. With the reduced consumption of the preparation, the aerosol method makes it possible to accomplish decontamination of the air and surfaces, which is one of its most important advantages over the damp-chemical method (Lagert, 1956; Grishina et al., 1965 and others). Hydrogen peroxide aerosols have a virucidal effect. Serebryakova et al., (1969) achieved decontamination in 45 minutes in an experimental chamber, purifying air which had been artificially infected with flu virus, consuming 0.105 g/m^3 of hydrogen peroxide, determined on the basis of the active substance (AS)*¹ with an air temperature of 15 to 20°

*¹In the following, the consumption of aerosol is given on the basis of the AS.

and a relative humidity of 50 to 70%. Slobodenyuk and Karpukhin (1970) inactivated the A2 flu virus in the air and on the surfaces of an experimental chamber using hydrogen peroxide aerosols and consuming 20-40 mg/m³ of the preparation.

In view of the fact that the conclusions concerning the virusidal activity of aerosols of hydrogen peroxide are based only on experimental data, we resolved to test the effects of these aerosols on flu virus in an epidemic nidus. The work was performed in a pediatric ward during a period when there was an increase in flu cases. During the period from February to March 1970, 29% of the children were ill with flu.

Disinfection was performed in three separate groups and in an isolation ward where children sick with flu were located. In the two groups that served as controls the area was closed by means of an air-conditioning system.

There were 20 to 22 children in each group. The arrangement and size of the rooms for the groups were all the same: a play-room (180 m³) where the children spent most of the day and ate their food, a bedroom (120 m³) and auxiliary areas (48 m³).

The hydrogen peroxide aerosols were sprayed using the non-apparatus method simultaneously in 2-3 points in the room at a level 1.5-2 meters from the floor with the doors, windows and air vents closed. Following exposure lasting no more than 5 minutes, the area was filled with a fog of polydisperse aerosols, which remained 30 to 40 minutes in the air depending on the amount of atomized preparation.

In planning the disinfection of the air in the presence of human beings, we experienced difficulty in choosing the working consumption of the preparation. The maximum permissible concen-

tration of hydrogen peroxide in the air of living quarters has not been standardized and the permissible concentration of this substance in air of industrial locations has been evaluated in different ways. The Institute of Labor Hygiene and Professional Diseases of the USSR Academy of Medical Sciences considers a concentration of hydrogen peroxide in work areas of 0.001 g/m^3 to be permissible (according to the AS), while in the USA the concentration is 0.0014 g/m^3 for a 90% preparation. The All-Union Institute of Disinfection and Sterilization considers that warm-blooded animals will not be harmed when the air contains 0.055 g of hydrogen peroxide per m^3 , used once a day for 10 days. Taking these data into account, under the conditions in this pediatric department we used hydrogen peroxide for disinfecting /139 the air at the rate of $0.020\text{--}0.035 \text{ g/m}^3$ (on the basis of the AS) on one occasion for a day. In the majority of experiments the children were brought into the treated areas 30-40 minutes following disinfection, when the larger aerosol particles had settled out and the air had become transparent. In only a few cases were the aerosols used in the presence of the children.

Disinfection was carried out at an air temperature of $20\text{--}22^\circ$ and a relative humidity of 21-29%, and after spraying the aerosol it dropped 2 to 7% for 60-70 minutes. The high degree of dryness of the air may have been of help to the lower resistance of aerodisperse systems.

The rooms were ventilated after collecting a sample of the air.

The virusidal effect of aerosols of hydrogen peroxide was determined by studying the air to find flu viruses. Samples were taken prior to the disinfection of the area, 40 minutes afterward and three hours following dispersal of the aerosols, on the basis of the fact that inactivation of flu A virus requires 45

minutes (Sherbryakova et al., 1969), which is also the sedimentation time for most of the aerosol from the air in the room. Prior to disinfection the children were kept in the room for 2 1/2 to 3 hours. Air samples (220 meters in 14 minutes) were collected with a bubbling apparatus connected to a vacuum cleaner. The air passed through 5 ml of a mixture composed of 4 ml normal allantoic fluid (or sugar bouillon) and 1 ml of a solution of sodium hyposulfite. After the sample was collected, 100 units of penicillin and streptomycin each were added to 1 ml of the mixture. The samples were frozen once, thawed and centrifuged. The supernatant fluid (0.2 ml) was used for infecting 10-day chick embryos (in the amnion) and incubated at 37° for 48 to 72 hours. The passage of the material from the infected embryos was conducted three times. The presence of the flu virus was checked after each passage on the basis of the results of the hemagglutination reaction (HAR) with allantoic and amniotic fluids. The HAR was established by the generally accepted method with 0.4 ml of fluid and 1% suspension of chick erythrocytes in a dilution of 1:2. The samples with positive results were typed in the hemagglutination inhibition reaction (HAIR) with sera, either as type A or B viruses. At the same time, the substrate through which the air samples had passed, or the allantoic fluid following a single passage in chick embryos, was used to infect white mice intranasally. The presence of the flu virus was determined on the basis of the specific irritation of the lungs in the mice and the results of the HAR with a suspension of lung tissue.

The virus was found in 12 out of the 27 air samples that were collected prior to disinfection of the group in the rooms and in the isolation ward, where those who were sick with flu were located (Table 1). It was identified by the All-Union Regional Center for Influenza following the third passage in embryos (hemagglutination titer 1:512 - 1:64) as Type A2 flu virus (Hong Kong-68

strain*). None of the 24 air samples that were collected 40 minutes following spraying of 4.3 to 5.4 ml of aerosol mixture ($0.020-0.031 \text{ g/m}^3$ according to the AS), showed any flu virus. After 3 hours, in these same areas, after children had been in them 1 1/2 hours, only 1 sample showed a virus of this type with a hemagglutination titer of 1:2. In two cases there were insignificant changes in the lungs of the mice.

The virucidal effects of using hydrogen peroxide in the isolation ward were particularly convincing, where the disinfection was carried out in the presence of flu patients. From the air samples that were collected here prior to the spraying of the aerosols, the type A2 flu virus (Hong Kong-68)** was isolated; a virus of the same type was found in smears from the nasopharynges of the patients. 40 minutes and 3 hours following disinfection of the ward by hydrogen peroxide aerosols at the rate of 0.022 mg/m^3 (according to AS), no virus was found in any of the 7 samples. Out of the 22 samples that were collected in the control groups in the course of the day, the flu virus was found in 6 (Table 2). /140

In evaluating the results of these experiments, we can conclude that the aerosols of hydrogen peroxide have a clear virucidal effect with a preparation consumption of 0.020 to 0.031 g/m^3 (according to AS) even under conditions that made disinfection difficult: flu patients present in the rooms and low relative air humidity. The virucidal activity of the aerosols of hydrogen peroxide following a single spraying lasted 3 hours.

It must be kept in mind that in order to achieve inactivation of flu virus (Type A2, Hong Kong-68 strain) under conditions of

* Recorded as No. 1-A2 Hong Kong-68 (USSR 0291/70), No. 2-A2 Hong Kong-68 (USSR 0292/70) and No. 3-A2 Hong Kong-68 (USSR 0293/70).

** Recorded in the All-Union Regional Center of the USSR for Influenza as No. 0291/70.

TABLE 1. EFFECT OF HYDROGEN PEROXIDE AEROSOLS ON FLU VIRUS
IN THE DISINFECTION OF THE AIR IN ROOMS

Area disinfected	Object of investigation	Presence of virus			Consumption of aerosols (in g/m ³)	Presence of virus in air samples following disinfection					
		Samples of virus prior to disinfection				After 40 minutes			After 3 hours		
		HAR with chick embryos	Infection of mouse lungs	HAR with suspension of mouse lungs		HAR with chick embryos	Damage to mouse lungs	HAR with mouse lung suspension	HAR with chick embryos	Damage to mouse lungs	HAR with mouse lung suspension
Group No. 7 isolation ward	Air	+	—	—	0.020	—	—	—	—	—	—
	Smears from throat	+	+	+	—	—	—	—	—	—	—
Group No. 10 isolation ward	Air	+	+	+	0.021	—	—	—	—	—	—
	"	+	+	+	0.021	—	—	—	—	—	—
Group No. 5 isolation ward	"	+	+	—	0.022	—	—	—	—	—	—
	"	+	+	—	0.022	—	—	—	—	+	—
isolation ward	"	+	+	+	0.022	—	—	—	—	—	—
	Smears from throat	+	+	+	—	—	—	—	—	—	—
Group No. 6	Air	—	+	+	0.024	—	—	—	—	+	—
	"	+	+	+	0.025	—	—	—	—	—	—
	"	+	+	+	0.026	—	—	—	—	—	—
	"	+	+	—	0.027	—	—	—	+	—	—
	"	+	—	—	0.031	—	—	—	(1:2)	—	—
Group No. 10	"	+	0	0	0.027	—	—	—	—	0	0

Note: + presence of virus; ± questionable result; 0 sample not investigated

TABLE 2. SEPARATION OF FLU VIRUS FROM AIR SAMPLES
IN AREAS OCCUPIED BY CONTROL GROUPS

Area studied	Presence of virus in air		
	HAR with chick embryos	Damage to mouse lungs	HAR with mouse suspension lung
Group No. 8	+	+	+
	+	+	—
	+	+	+
	+	+	—
Group No. 11	—	+	—
	+	+	+

Note: Symbols same as in Table 1.

practical disinfection, 4 times less hydrogen peroxide was used (according to AS) than in the experiments of Serebryakova et al., (0.105 g/m³). We feel that this has to do with the lower concentration of a virus against which the disinfection was carried out in the epidemic nidus than that with which these authors experimented. It is also possible that the Type A flu virus (PR₈ strain) which they employed was more resistant to the disinfectant. In the experiments of Slobodenyuk and Karpukhin with A2 flu virus, the consumption of hydrogen peroxide which ensures virucidal effects (20-40 mg/m³) coincided with that which we established (20-30 mg/m³).

In those few cases when the aerosols were used in the presence of children, with disinfectant consumption at the rate of 0.020 and 0.027 g/m³ (according to AS), and also when the children were brought into the room after 30 to 40 minutes had elapsed following

its disinfection, we failed to notice any signs of intoxication /141
in them. The personnel who took part in the performance of the experiment and were subjected to a more massive effect in the course of 3 to 4 hours, when the preparation was used in amounts of 0.020-0.027 g/m³ (according to AS), there was a mild irritation of the mucous membranes of the eyes and upper respiratory pathways. When the aerosol concentration was increased to 0.029-0.035 g/m³ (according to AS) nausea, vomiting, headache and severe irritation of the mucous membranes developed. These symptoms of intoxication passed after 1 to 2 hours without any medical aid. Masks made of 4-layer gauze did not provide protection against the effect of the polydispersed aerosols of hydrogen peroxide and were destroyed after repeated use.

We did not observe any noticeable changes in color or transparency of the medical smocks, handkerchiefs, parts made of cloth or toys that were in areas treated with hydrogen peroxide aerosols.

What we have said above regarding tolerance of hydrogen peroxide aerosols is largely responsible for the possibility of using them under practical conditions. In our opinion, it is only possible to use these aerosols for the disinfection of areas where human beings are always present only in cases of extreme necessity. It is most advisable to use the aerosol to disinfect the air of an area when persons are in it for a short period of time. Repeated use of hydrogen peroxide aerosols in concentrations which have a disinfecting effect, in the presence of a given group of persons, is felt by us to be inadmissible from the toxicological point of view. Hydrogen peroxide aerosols may be used for disinfection of boxes of sampling equipment in infection stations, pediatric wards used for prevention and treatment, bacteriological laboratories, and for the disinfection of sanitary means of transport.

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